

## REMARKS

### Status of the Claims

Claims 1 and 21 have been amended without prejudice to or disclaimer of the subject matter therein to recite "...wherein said first NP and said second NP are brought into closer proximity on said circular DNA molecule relative to said contiguous DNA segment...." Support for the amendments can be found in the specification on page 3, lines 20-21. Claims 1-18 and 21-22 are now pending.

The Examiner's comments are addressed below in the order set forth in the Office Action dated July 7, 2003.

### The Rejections of the Claims Under 35 U.S.C. § 103 Should Be Withdrawn

Claims 1-16, 21, and 22 stand rejected under 35 U.S.C. § 103 over Li *et al.* (1998) *BioTechniques* 25:358-361 in view of Patel *et al.* (1991) *Nucleic Acids Res.*, 19:3561-3567 and Michalatos-Beloin *et al.* (1996) *Nucleic Acids Res.* 24:4841-4843. This rejection is respectfully traversed because a *prima facie* case of obviousness has not been established.

To establish a *prima facie* case of obviousness (1) there must be some suggestion in the reference or knowledge generally available to one of ordinary skill in the art to modify the reference or combine the references; (2) there must be a reasonable expectation of success; and (3) the prior art reference(s) must teach or suggest all the claim limitations. MPEP § 2143.

The requirement for the establishment of motivation to combine references is central to the obviousness inquiry, and has been explained by the Federal Circuit in the following manner:

When patentability turns on the question of obviousness, the search for and analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness. *See, e.g., McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1351-52, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001) ("the central question is whether there is reason to combine [the] references," a question of fact drawing on the Graham factors).

"The factual inquiry whether to combine references must be thorough and searching." *Id.* It must be based on objective evidence of record. This precedent has been reinforced in myriad decisions, and cannot be dispensed with. *See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124-

25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000) ("a showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential component of an obviousness holding'"') (*quoting C.R. Bard, Inc., v. M3 Systems, Inc.*, 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998)); *In re Dembicza*k, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) ("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references."); *In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (there must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant); *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988) ("teachings of references can be combined only if there is some suggestion or incentive to do so.") (emphasis in original) (*quoting ACS Hosp. Sys., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)).

*In re Lee*, 277 F.3d 1338, 61 U.S.P.Q.2d 1430, 1433-4 (Fed. Cir. 2002).

In the present rejection, the objective evidence of record does not support a conclusion that one of skill in the art would have been motivated to combine Li *et al.*, Patel *et al.*, and Michalatos-Beloin *et al.* The lack of motivation for the combination of references asserted in the Office Action becomes clear when one considers the teachings of each reference without the benefit of hindsight. Applicants have summarized the teachings of each reference in the following paragraphs.

Li *et al.* (1998) BioTechniques 25:358-361

Li *et al.* teaches a multi-step method for determining the haplotype of an individual by allele-specific PCR amplification (ASPA), where the sequences containing the polymorphism(s) are (1) found on highly homologous genes and (2) the polymorphisms are separated by a distance too great (roughly 30-40 base pairs) for a single PCR primer to be designed. The method overcomes the inherent difficulties encountered when attempting to use single-step ASPA for determining the haplotype of an individual at one locus when highly homologous genes exist. In particular, when a single step ASPA technique is applied in a situation in which there are highly homologous genes not only are amplification products generated from the desired gene, but spurious amplification from the homologous genes occurs as well. This

renders the results extremely difficult to interpret. See Li, page 358, paragraph spanning columns 2-3.

To overcome these difficulties, Li *et al.* first amplify the region containing the polymorphism using at least one *gene-specific* primer. *Id.* The resulting PCR products are then circularized via intramolecular ligation and a single round of allele specific inverse PCR (ASIP) is carried out. Inverse PCR is utilized because the specific T at the upstream polymorphism is too distant (30-43 base pairs) from the other polymorphisms for a single primer to be designed. *Id.*, col. 2, first paragraph and the paragraph spanning pages 360-1.<sup>1</sup>

Patel *et al.* (1991) Nucleic Acids Res., 19:3561-3567

Patel *et al.* teaches a multi-step method of determining the haplotype of an individual. First, a restriction digest is carried out on genomic DNA isolated from an individual to be haplotyped. Second, the DNA fragments generated from the restriction digest are circularized using a DNA ligase. Third, the fragment is re-linearized utilizing a different restriction enzyme. Fourth, the haplotype is assessed by PCR utilizing Amplification Refractory Mutation System primers. Unlike Li *et al.*, Patel *et al.* does not address problems arising from the existence of homologous genes.

Michalatos-Beloin *et al.* (1996) Nucleic Acids Res. 24:4841-4843

Michalatos-Beloin *et al.* teaches a one-step method of haplotype differentiation based on the use of allele-specific primers and the differing size or presence of PCR products depending on the haplotype structure. Unlike either Li *et al.* or Patel *et al.*, no circularization of the long-range PCR products is required, nor would such circularization be of any benefit for this method.

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<sup>1</sup> The alleles discussed in Li *et al.* are as follows:

- M<sup>G</sup>: G.....C...GT
- M<sup>T</sup>: T.....C...GT
- N: T.....T...AG

Applicants' method

In contrast to the references cited in the Office Action, Applicants have disclosed and claimed a method for determining the haplotype structure of a contiguous DNA segment comprising a first nucleotide polymorphism (NP) and a second NP separated by at least 200 nucleotides. The method comprises the steps of, first, obtaining a DNA sample comprising said contiguous DNA segment and, second, using the DNA sample as a template for polymerase chain reaction (PCR) amplification of a DNA fragment comprising said contiguous DNA segment. The third step involves ligating the ends of said DNA fragment to each other so as to produce a circular DNA molecule, "*wherein said first NP and said second NP are brought into closer proximity on said circular DNA molecule relative to said contiguous DNA segment,*" after which the haplotype is determined. Unlike Li *et al.*, Applicants' method brings the NPs into closer proximity on the circular DNA molecule relative to the contiguous DNA segment used for the first round of PCR. Although Applicants traverse the present rejection, the italicized language (above) has been added to Applicants' claims to highlight this effect.

By assessing the references without hindsight reliance on Applicants' disclosure, several crucial differences between the approaches taught by the references and Applicants' own teachings become clear. This is explained in detail in the following paragraphs.

As discussed above, the *only* problem involving distance described in Li *et al.* was the problem caused by polymorphisms that are closely linked (within 30-43 bases apart), but too distant to allow the design a *single* haplotype specific primer. Li *et al.*, page 358, col. 2, first paragraph. Li *et al.* solved this problem by circularizing the products of their initial PCR step and using inverse PCR to haplotype the polymorphisms. Li *et al.* teach that circularization and allele-specific inverse PCR overcomes the difficulty posed by the 30-43 base pairs between the M<sup>T</sup> polymorphisms. See Li *et al.*, page 358, column 2, first paragraph and the paragraph spanning pages 360-361, stating "[a]lthough primers specific to M<sup>G</sup> and N alleles can be designed, the M<sup>T</sup>- (and N-) specific nucleotide T in intron 1 is located too far (30-43 bp upstream) from the three M- (M<sup>G</sup> and M<sup>T</sup>) specific bases in exon 2 to design a single M<sup>T</sup>-specific primer."

Li *et al.* discuss their use of allele-specific inverse PCR (ASIP) as a single-step solution to this problem in the closing paragraph as follows.

This technique [inverse PCR], which involves ligation of separated regions at the ends of a sequence (a restriction fragment or a PCR product), can also be applied to closely linked polymorphisms. Haplotypes consisting of heterozygous polymorphisms have been determined by pedigree analysis. However, using inverse PCR, the linked polymorphisms can be analyzed by a single procedure using allele-specific primers. In this study, M<sup>G</sup>, M<sup>T</sup>, and N alleles were regarded as haplotype alleles and analyzed by ASIP. Although these alleles can be typed by allele-specific nested PCR following GPA-specific PCR, ASIP, rather than nested PCR, can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR.

Li *et al.*, column spanning pages 360-1.

The Office Action makes much of the final sentence in the reference, asserting that it supports a conclusion that the circularization of Li *et al.* should be combined with long-range PCR of Michalatos-Beloin *et al.* In order to support such a conclusion, however, one would have to interpret the phrase “too long to be amplified by PCR” to mean “too long to be amplified by standard PCR but not too long to be amplified by long range PCR (LR-PCR).” The only apparent reason for this interpretation is to provide a rationale for combining Li *et al.* with Michalatos Beloin *et al.* A more plausible interpretation is that LR-PCR was contemplated as a form of PCR when the statement was made considering that (a) LR-PCR was a form of PCR known well before publication of the Li article in 1998, as evidenced by the Michalatos Beloin article itself, which was published in 1996, and (b) the issue addressed in the statement made by Li is the outer limits of PCR in terms of length of DNA amplified, an issue which clearly elicits consideration of LR-PCR. In other words, Li *et al.*’s statement was related to a situation in which PCR could not be utilized at all. If this more plausible interpretation is adopted, then the rationale for combining Li *et al.* with Michalatos Beloin *et al.* evaporates.

The Office Action further states: “An ordinary practitioner would have been motivated to use long range PCR to prepare the template for the method of Li in order to extend the range of detection of polymorphisms *in order to solve the problem of Li that there are 'polymorphisms separated by a distance that is too long to be amplified by PCR* (see page 361, column 1).” The quote is clearly taken out of context when one considers the following teachings of Li *et al.*:

- Li *et al.* teaches haplotyping allele specific polymorphisms separated by a distance a few tens of base pairs. Li *et al.*, page 358, col. 2, line 11. The problem that results from this separation is that the M<sup>T</sup> allele cannot be amplified by PCR using a single allele-specific primer.
- Li *et al.* teaches a method that does not bring the relevant polymorphisms into closer proximity. See Li *et al.*, Figure 1. Thus, counter to the Office Action's assertion, the method of Li *et al.* provides *no* advantage for determining the haplotype of polymorphisms separated by a distance of 200 base pairs or more.
- Li *et al* teaches the use of inverse PCR after ligation, which would negate any advantage of bringing linked polymorphisms in closer proximity IF the polymorphisms actually were brought into closer proximity.
- Li *et al.* explains that their use of allele-specific inverse PCR allows the linked polymorphisms to be analyzed in a *single* procedure using allele-specific primers. Li *et al.*, sentence spanning pages 360-61.

When considered in light of the actual teachings of Li *et al.*, it is clear that the distance problems discussed in Li *et al.* were those caused by the difficulty of designing a single primer that covers the polymorphisms of the M<sup>T</sup> allele, i.e., 30-43 base pairs. In contrast, Applicants' claimed method is directed to determining the haplotype of a "first nucleotide polymorphism (NP) and a second NP separated by at least 200 nucleotides[.]" See Claim 1.

Despite the teachings of Li *et al.*, the Office Action implies that Li *et al.* had identified the determination of haplotyping sequences separated by over 200 base pairs as problematic. Notably, the Office Action fails to make *specific* findings to support this implication. Instead, it relies upon conclusory statements such as "Li recognizes the problem in that some haplotypes are too distant to be amplified by standard PCR" and "Michalatos-Beloin solves the problem using long range PCR." However, the only *distance-related* problem Li *et al.* describe was caused by the 30-43 base pair separation between the polymorphisms of the M<sup>T</sup> allele, a distance too long for the design of a *single* PCR primer. To conclude that the final sentence of Li *et al.* referred to problems encountered by polymorphisms separated by 200 base pairs or more, one would have to assume that (1) Li *et al.* would wait until the final sentence of their article to

identify the problem and (2) suggest that allele-specific inverse PCR offered a solution *without offering a single explanatory sentence*. One would only make these assumptions when considering Li *et al.* in light of *Applicants' own* disclosed method, i.e., generating a PCR product from a contiguous DNA comprising two nucleotide polymorphisms separated by more than 200 bases and circularizing the template such that the polymorphisms are brought into closer proximity on the circular DNA molecule relative to the contiguous DNA.

The present rejection is insufficient because (1) it lacks the required findings regarding Li *et al.* and (2) is based upon the impermissible hind-sight application of Applicants' own invention. It should be withdrawn accordingly. *See In re Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000)(specific findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected the components of the claimed invention); *see also Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 796 F.2d 443 (Fed. Cir. 1986)(a single line in a prior art reference should not be taken out of context and relied upon with the benefit of hindsight to show obviousness).

If additional support exists for the Office Action's conclusion that Li *et al.* referred to problems encountered with polymorphisms separated by distances of 200 base pairs or greater, Applicants request that the particular reference be cited or that the Examiner provide an affidavit as required by 37 CFR 1.104(d)(2).

The Office Action also asserts that Patel *et al.* teaches "inverse PCR methods such as those used by Li *et al.* can be applied to haplotype sequences up to 10 kb apart...." As explained above, the inverse PCR step taught by Li *et al.* provides no advantage for haplotyping sequences "up to 10 kb apart" because the circularization step of Li *et al.* does not bring the polymorphisms in closer proximity. The required showing for the Office Action's combination of Li *et al.* with Patel *et al.* has not been established.

The Office Action contends that the motivation to combine Li *et al.* with Michalatos-Beloin *et al.* can also be found in the following quote from Michalatos-Beloin *et al.*: "The ability to isolate hemizygous DNA segments readily from heterozygous genomes via molecular haplotyping will provide the accuracy necessary in these diverse applications' (page 4867, column 2)." However, the language quoted by the Office Action does not provide the motivation

to combine Michalatos-Beloin *et al.* with Li *et al.* Rather, in the quoted passage Michalatos-Beloin *et al.* promotes the usefulness of their own one-step PCR method of haplotyping, a method that would not benefit from any additional manipulations. Rather than supporting the combination of Li *et al.* with Michalatos-Beloin *et al.*, the quoted passage teaches away from its modification or combination with any other method.

The Office Action also states that Patel *et al.* provides motivation to apply the method of Michalatos-Beloin *et al.* to that of Li *et al.* because Patel *et al.* "teaches that haplotyping using inverse PCR is desirable on long segments, even haplotypes separated by more than 10,000 nucleotides...." However, Michalatos-Beloin *et al.* itself teaches the desireability of haplotyping over long distances *and also* teaches that a one-step method of haplotyping without additional manipulations such as ligation, etc., is the desireable solution. In the Michalatos-Beloin method, haplotyping is *conducted* by a single-step PCR protocol and one need only analyze the PCR products of the Michalatos-Beloin method to determine the haplotype of the source DNA. Michalatos-Beloin *et al.* teaches away from its combination with methods that require additional steps to analyze the haplotype of a sequence of interest. Accordingly, the art worker would be motivated to utilize Michalatos-Beloin *instead* of Li *et al.* (or Patel *et al.*, for that matter), not to combine the two.

With respect to claims 21 and 22, the Office Action asserts that "Li teaches a DNA sequence immediately adjacent to the 5' and 3' NPs which is less than 50 bases long." This statement is misleading because it analogizes the second PCR step of Li *et al.* to the first step of claim 21. Claim 21 recites:

A method for determining the haplotype structure of a contiguous DNA segment comprising a first nucleotide polymorphism (NP) and a second NP separated by at least 200 nucleotides, said method comprising:

- (a) obtaining a DNA sample comprising said contiguous DNA segment, wherein the DNA segment further comprises
  - a DNA sequence immediately 5' to the first NP that encompasses an annealing site for a primer and
  - a DNA sequence immediately 3' to the second NP that encompasses an annealing site for a primer;
- (b) using said DNA sample as a template for polymerase chain reaction (PCR) amplification utilizing said primers of a DNA fragment comprising said contiguous DNA segment;

- (c) ligating the ends of said DNA fragment to each other so as to produce a circular DNA molecule, wherein said first NP and said second NP are brought into closer proximity on said circular DNA molecule relative to said contiguous DNA segment; and
- (d) determining the haplotype of said first NP and said second NP.

In claim 21, it is the *first step* of PCR in which the annealing site for the PCR primers is immediately 5' or 3' to a NP. In contrast, the first PCR step of Li *et al.* uses *gene-specific* primers that *do not* anneal immediately 5' and 3' to the subject nucleotide polymorphisms. If the Office Action's analogy were accurate, the PCR product generated by the first step of Li *et al.* would be less than 143 base pairs (50 base pairs 5' + 50 base pairs 3' + (30 to 43) base pairs between the upstream and downstream polymorphisms). However, the PCR product of the first step of Li *et al.* is greater than 300 base pairs. Thus, Li *et al.* fails to teach or suggest an initial PCR step in which the primer annealing sites are immediately 5' or 3' to the polymorphisms.

For all these reasons, no motivation to select the components of Applicants' method has been established. Rather, each reference teaches a different method intended to overcome different problems encountered by the art. The present rejection should therefore be withdrawn. *See In re Gordon*, 221 U.S.P.Q. 1125 (Fed. Cir. 1984)(mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification).

The present rejection should also be withdrawn because there is no evidence in the record that the combination of either Li *et al.* or Patel *et al.* with Michalatos-Beloin *et al.* would yield a method operable for its intended purpose. For instance, the method of Michalatos-Beloin *et al.* is intended to provide allele discrimination by long-range PCR using allele specific PCR primers that are not designed for circularization. The system of Michalatos-Beloin *et al.* is not compatible with the additional manipulations found in the other references and it therefore teaches away from the combination of references asserted in the Office Action. *Tec Air Inc. v. Denso Manufacturing Michigan Inc.*, 52 U.S.P.Q.2d 1294, 1298 (Fed. Cir. 1999)(a combination of references that would produce a device inoperable for its intended purpose teaches away from combining same).

Based upon this analysis of the prior art, it is clear that the teaching, motivation, or suggestion to select and combine the references has not been demonstrated. To summarize, nothing in the record demonstrates that one of skill in the art would have had the motivation to combine Li *et al.* with Michalatos-Beloin *et al.* or Patel *et al.* Indeed, the present rejection constitutes a hindsight-based obviousness determination. For all of these reasons, Applicants respectfully request that the rejection be withdrawn.

Claim 17 is rejected under 35 U.S.C. § 103 over Li *et al.* in combination with Patel *et al.* and Michalatos-Beloin *et al.* in further view of Krynetski *et al.* (1995) *Proc. Natl. Acad. Sci.*, 92:949-953. Applicants respectfully traverse.

As described above, there is no motivation to combine Li *et al.* with Patel *et al.* and Michalatos-Beloin *et al.* Krynetski *et al.* merely teaches a point mutation of the TPMT gene and does not satisfy the deficiencies of the primary references. Consequently, the motivation to combine these four references has not been established and the rejection should be withdrawn.

Claim 18 is rejected under 35 U.S.C. § 103 over Li *et al.* in combination with Patel *et al.* and Michalatos-Beloin *et al.* in further view of Martin *et al.* (2000) *Am. J. Hum. Genet.*, 67:383-394. Applicants respectfully traverse.

As already explained, there is no motivation to combine Li *et al.* with Patel *et al.* and Michalatos-Beloin *et al.* Martin *et al.* teaches SNPs in the region surrounding the APOE gene but does not satisfy the deficiencies of the primary references. Accordingly, the motivation to combine these four references has not been established and the rejection should be withdrawn.

## **CONCLUSION**

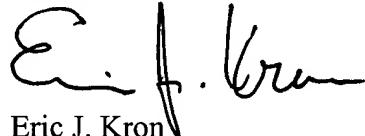
In view of the aforementioned amendments and remarks, Applicant respectfully submits that the objection to the specification and the rejections of the claims under 35 U.S. C. § 103 are overcome. Further, the rejection should not be applied to the claims as amended. Accordingly,

Applicant submits that this application is now in condition for allowance. Early notice to this effect is solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,



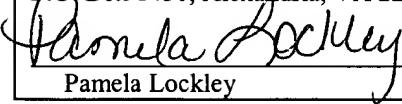
Eric J. Kron  
Registration No. 45,941

**Customer No. 29312**

**ALSTON & BIRD LLP**  
Bank of America Plaza  
101 South Tryon Street, Suite 4000  
Charlotte, NC 28280-4000  
Tel Raleigh Office (919) 862-2200  
Fax Raleigh Office (919) 862-2260

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Pamela Lockley